T4 RNA Ligase 1

User's Instruction

Description

T4 RNA Ligase 1 is a ligation enzyme for catalyzing the ligation of a 5′ phosphoryl-terminated nucleic acid donor to a 3′ hydroxyl-terminated nucleic acid acceptor through the formation of a 3′ → 5′ phosphodiester bond. This enzyme requires ATP for activity. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates.

Kit Contents

	1 KU
1. T4 RNA Ligase 1 (10 U/μl)	100 μl
2. 10×T4 RNA Ligase Buffer	250 μl
3. 10mM ATP	100 μl
4. 50% PEG 8000	500 μl

**1 × T4 RNA Ligase Buffer：50mM Tris-HCl (pH 7.5), 10mM $MgCl_2$, 1mM DTT.*

Applications

- Joining single-stranded RNA or DNA fragments
- Labeling of 3'-termini of RNA with 5'-[32P] pCp
- Inter- and intramolecular joining of RNA and DNA molecules
- Synthesis of single-stranded oligodeoxyribo-nucleotides
- Incorporation of unnatural amino acids into proteins

Unit Definition

One unit is defined as the amount of enzyme that converts 1 nmole of 5′-[32P]rA16 into a phosphatase-resistant form in 30 min at 37°C.

Protocol

Joining single-stranded RNA or DNA fragments

1. Set up the reaction as the following table (take 20 μl per well as an example):

Reagent	Volume
RNA or ssDNA	0.2-2 pmol
10×T4 RNA Ligase Buffer	2 μl
50% PEG 8000	5~10% (wt/vol)
T4 RNA Ligase1 (10 U/μl)	1 μl
10mM ATP	2 μl
ddH_2O	Up to 20 μl

2. The reaction time is 30-60 min at 37°C, or 1-2 h at 25°C.

3. Terminate the reaction at 65°C for 15 min.

RNA cyclization

1. Set up the reaction as the following table (take 20 μl per well as an example):

Reagent	Volume
RNA	10 μM
10×T4 RNA Ligase Buffer	2 μl
50% PEG 8000	5~10% (wt/vol)
T4 RNA Ligase1 (10 U/μl)	1 μl
ATP	20-50 μM
RNase Inhibitor	0.5 μl
ddH_2O	Up to 20 μl

2. The reaction time is 30-60 min at 37°C, or 1-2 h at 25°C.

3. Terminate the reaction at 65°C for 15 min.

Storage

Minimum shelf life is 3 years under -20°C.

Additional Notes

- 5 '- phosphorylation or preadenylation is required for the binding of ssDNA and ssRNA.
- The enzyme can not ligate double-stranded DNA or RNA.
- Adding final concentration of 5-10% PEG 8000 or increasing incubation time can improve the ligation efficiency.